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DIVERSITY OF BACTERIA AND FUNGI AND PHAGOCYTIC PROPERTIES OF NEUTROPHILS IN SOME RESPIRATORY DISEASES

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Abstract

Respiratory diseases are considered serious diseases that affect all age groups and can lead, in most cases, to death. This study aimed to investigate the bacterial and fungal causes of some respiratory infections and the relationship between the phagocytic activity of polymorphonuclear cells and respiratory tract infection.146 clinical samples of sputum were collected from 50 patients with chronic tuberculosis, and pharyngeal swabs from 96 patients with respiratory infections other than tuberculosis, including chronic bronchitis (22 patients), allergic (66 patients), and asthma (5patients) pneumonia (3 patients). These samples were collected from patients referred to the Specialized National Centre for Chest and Respiratory Diseases in Baghdad for the period between 2018and 2019. It has been found that out of the total 146 patients, the number of infected males is 76 (52.1 %) and 70 females (47.9%). 174 bacterial isolates were isolated and identified, as well as it was noted that the percentage of respiratory infections caused by gram-positive bacteria was higher (66.1%) compared to the respiratory infections caused by gram-negative bacteria (33.9%), As for fungal infections, from 140 isolates showed that the percentage of respiratory infections caused by fungi was the highest (70.9%) compared to respiratory infections caused by yeasts (29%). The current study indicated that respiratory tract infections stimulated the phagocytic activity of polymorphonuclear cells for all age groups.

Keywords: Respiratory Infections, Tuberculosis, Fungal Infections, Neutrophils, Bacterial Infections.

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Introduction

Respiratory infections represent one of the most important infections that affect humans, and this infection usually targets both the upper respiratory tract and the lower respiratory tract. The lower respiratory infection is the most dangerous, as it targets the lungs, bronchi, and bronchi. Infections of the respiratory tract and its appendages constitute a major problem, and prescriptions for their treatment with antibiotics amount to 75% of all infections. [1]. Respiratory infection occurs in several ways, including bacterial infection, virus infection, fungal infection, and others. The ways of transmission of infection vary, including what happens as a result of infection from infected people through droplets, or the use of patients' tools such as towels, or through what is known as a hospital infection, a Nasocomial infection[2].

Many pathogens are involved in causing infections, including viruses as well as bacteria, many of which can be treated by antibiotics, and to a lesser extent, fungi and parasites [3]. The most common disease that affects the lower respiratory tract is Pneumonia, which is considered one of the diseases dangerous to children's health and affects about 80% of children with pneumonia and 10% of adults. It is caused by bacterial infections caused by *Haemophilus influenza*, *Streptococcus pnenmoniae* [4,5]. Tuberculosis is one of the most dangerous bacterial infections that occur due to *Mycobacterium tuberculosis*. It represents one of the diseases that target the lungs directly, and this disease appears in more than one form as it enters the latency phase and the tuberculosis bacilli disappear in the scab. The importance of this disease comes from the fact that the danger it poses to the patient's life, through tissue destruction in the lungs, and through the immune weakness, that occurs in the patient's immune system. This, in its turn, facilitates the process of invasion and settlement of other pathogens to cause infection in healthy people [6]. The fungi are the other cause of respiratory infections, and the most important types of fungi isolated from respiratory infections are *A.terrus*, *Penicillium spp.* , *C.albicans* , *A.niger*, *A.fumigatus* , *A.flavus* and others[7].

Systemic and local defense mechanisms are effective in the respiratory system, and the respiratory system is exposed to infection mostly by inhalation, Cough and sputum are the distinguishing features of infection. There are many defense systems, mucous and non-mucous, such as 1- Nasal-associated lymphoid 2- Associated lymphatic tissue by trachea. 3 - Part of the auricular mucosa. 4 - Non-specialized humoral factors, such as alpha-tricin, lactoferrin, and lysosomes. 5- Mucosal immune response mediate cells. This is in addition to the cellular defense system of mononuclear (macrophages) while innate immunity is represented by a group of factors including physical and chemical barriers and phagocytes, while adaptive immunity is represented by humeral factors, lymphokines, and complement [8].

Materials and Methods

Sputum samples and pharyngeal swabs taken from patients with upper and lower respiratory tract infections were cultured on bacterial culture media represented by Nutrient agar, MacConkey agar, Blood agar, Mannitol agar, Chocolate agar and incubated at 37°C for (18-48) hours, and diagnosed using Gram stain and microscopic examination, biochemical tests were also used to isolate and diagnose bacterial isolates [9].To isolate and diagnose fungi from patients' samples, sputum samples, and pharyngeal swabs were planted on sabouraud medium and incubated at a temperature of 28 mm (5-7) days, and diagnosed using lactophenol dye and light microscopy [10]. The activity of neutrophils was measured using Nitroblue Tetrazolim reductase assay to determine their ability to phagocytosis [11].

Results and Discussion

The study included the examination of 146 patients suffering from respiratory tract infections, 50 patients suffering from chronic tuberculosis, and 96 patients suffering from other respiratory diseases, including chronic bronchitis (22) patients, allergy (66) patients, and asthma (5) patients and pneumonia (3) patients. The study also included identifying the pathogen most causing infection, and among the patients, an increase in the incidence of allergies and pulmonary tuberculosis appeared compared to other respiratory infections (Figure 1) and the reason for this is due to the increase in the antigens represented by bacteria, fungi, etc. Also, there and their easy entry into the respiratory tract or perhaps the reason is due to occupational factors in addition to the virulence factors of tuberculosis bacteria.

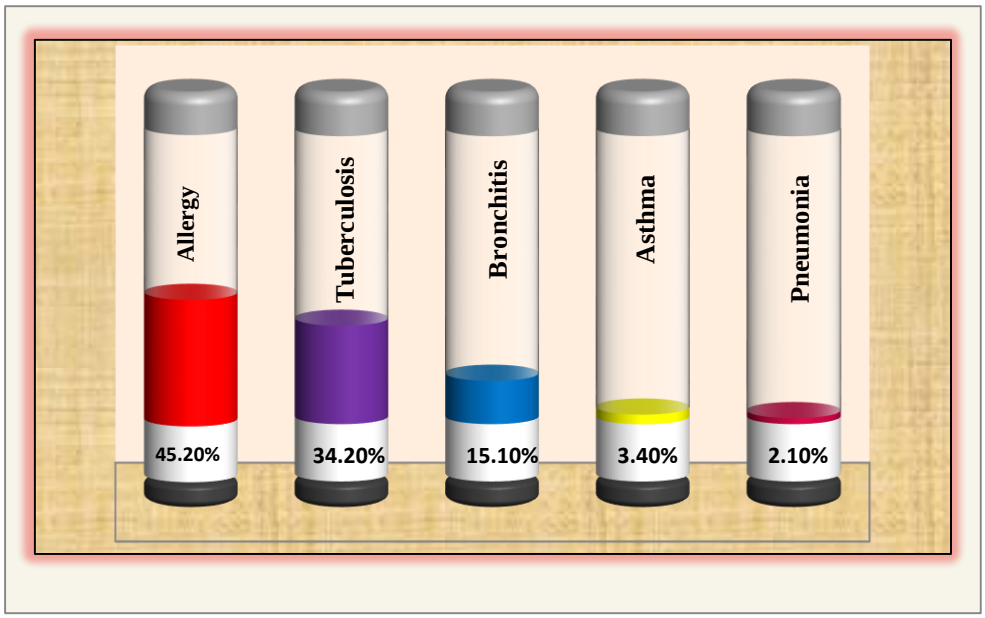

Figure 1: Number of patients according to the type of infection

The results showed that the bacteria that cause respiratory tract infections are more prevalent than the fungi that cause respiratory infections, as 174 bacterial isolates were isolated compared to 140 fungal isolates, as shown in Table (1). The presence and spread of bacteria in the upper respiratory tract may play a role in infection of the lower respiratory tract, especially in cases of a weak immune system. In addition, the study focused on the cold months of the year in which bacterial respiratory infections increase, especially tonsillitis, which is due to its ability to resist the effectiveness of antibacterial substances present in saliva in addition to their ability to adhere to the epithelial tissues of the oral cavity and respiratory tract[12].

Table 1: Distribution of bacterial and fungal infections in patients with respiratory tract infections

The type of disease	Isolates			
	Bacterial isolates		Fungal isolates	
	G +V	G --V	Yeasts	Molds
Tuberculosis	38	20	13	40
Chronic Bronchitis	18	10	6	10
Asthma	4	3	3	5
Allergy	53	26	19	42
Pneumonia	2	-	1	1
Total	115	59	42	98

The Relationship of Sex with Respiratory Infections

This paper studied the prevalence of respiratory infections between males and females in the patients under study. Itwas noted that the prevalence of respiratory infections was higher in males than in females, where it was found that out of the total 146 patients, the number of the infected males is 76 (52.1 %) and 70 females (47.9%), as shown in Figure (2). This may be due to the presence or spread of some bad habits in one sex over the other, including the prevalence of smoking and alcohol habits among males more than it is in females, which increases the rate of respiratory diseases[13] indicated that this may be due to the physiological and immunological differences between the sexes, which may facilitate or increase the rate of infection or it may be due to the immune susceptibility and the hormonal and functional differences between the sexes [14].

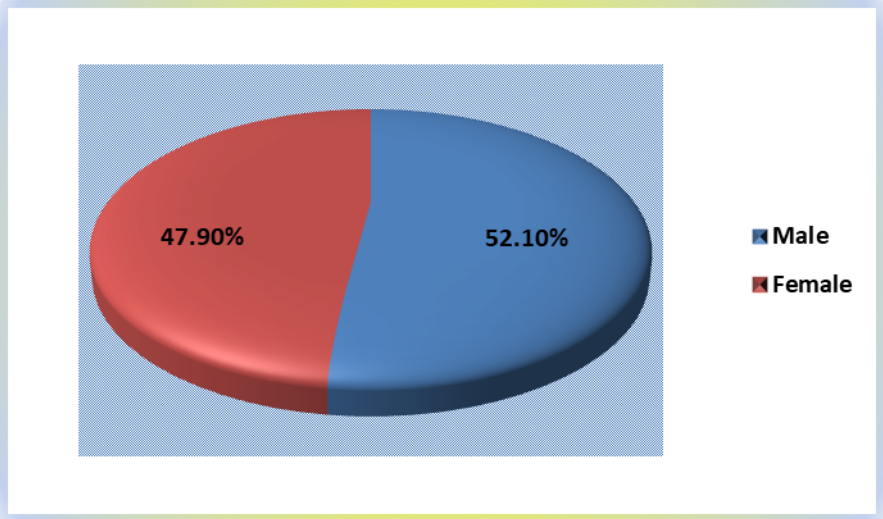

Figure 2: Distribution of respiratory infections according to the sex of the patient

Pathogens

1 - Bacterial infections:

The study showed that there can be more than one bacterial cause involved in respiratory infections, as more than one bacterial type was isolated at the same site of infection. This depends on the type of infection and the immune status of the host as well as

the effect that some pathogens have on the respiratory organs. It was noted that the percentage of respiratory infections caused by gram-positive bacteria was higher (66.1%) compared to the respiratory infections caused by gram-negative bacteria (33.9%), as shown in Table (2). This may be due to their ability to secrete some toxins and enzymes, and this plays a role in resisting phagocytosis in addition to its presence naturally in the respiratory tract. Explain the reason for this because these Gram-positive bacteria are naturally found in the nasopharyngeal region and their presence facilitates the process of their invasion of the lower respiratory tract, especially when there is a defect in the defense mechanisms that facilitate infection. When studying isolates of sputum from patients suffering from respiratory infections, it was found that they can resist the complement, and this is an important virulence factor that contributes to enhancing their ability to cause infection[15,16].

Type of Bacteria	Total of number		Allergy		Tuberculosis		Bronchitis		Asthma		Pneumonia	
	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%
G +V												
St. Pyogens	**31**	**26.9**	**16**	**51.6**	**10**	**32.2**	**3**	**9.6**	**1**	**3.2**	**1**	**.2**
St. Virdanse	**11**	**9.5**	**3**	**27.2**	**6**	**54.5**	**2**	**18.1**	**0**	**0**	**0**	
St. Pneumonia	**19**	**16.5**	**8**	**42.1**	**10**	**52.6**	**0**	**0**	**1**	**5.2**	**0**	
S. aureus	**38**	**33**	**17**	**44.7**	**9**	**23.6**	**9**	**23.6**	**2**	**5.2**	**1**	**.6**
S.epidermidis	**16**	**13.9**	**9**	**56.2**	**3**	**18.7**	**4**	**25**	**0**	**0**	**0**	
Total	**115**	**66.1**	**53**	**46.1**	**38**	**33**	**18**	**15.6**	**4**	**3.5**	**2**	**.7**
G –V												
M. Catarrhalis	**28**	**47.5**	**6**	**21.4**	**14**	**50**	**6**	**21.4**	**2**	**7.1**	**0**	
Ps. Aerogenosa	**21**	**35.5**	**15**	**71.4**	**5**	**23.8**	**1**	**4.7**	**0**	**0**	**0**	
K. Pneumonia	**3**	**5.1**	**2**	**66.6**	**1**	**33.3**	**0**	**0**	**0**	**0**	**0**	
H. Influenza	**2**	**3.3**	**1**	**3.8**	**1**	**5**	**2**	**20**	**1**	**33.3**	**0**	
N. Menengenities	**4**	**6.7**	**2**	**50**	**0**	**0**	**1**	**25**	**1**	**25**	**0**	
S. Macescenc	**1**	**1.6**	**0**	**0**	**0**	**0**	**1**	**100**	**0**	**0**	**0**	
Total	**59**	**33.9**	**26**	**44.1**	**20**	**33.8**	**10**	**16.9**	**3**	**5.1**		
Total Summation	**174**	**100**										

Table 2: Types of bacteria isolated from patients according to the type of infection.

2- Fungal infections:

140 fungal isolates were isolated at the same site of infections, and this depends on the type of infection and the immune status of the host, as well as the effect that some fungal causes have on the respiratory organs. As it is noted in table (3) that the percentage of respiratory infections caused by fungi was the highest. (70.9%) compared to respiratory infections caused by yeasts (29%) that the prevalence of infection with some fungal causes may be due to their presence and widespread in the environment and their possession of some virulence factors that enable them to cause infection and this may be due to the ability of fungi to grow in open cavities in the lungs of patients, especially those who suffer from tuberculosis, were the elements necessary for growth and high supply of free oxygen. The genus *A.niger* is the most prevalent isolate with a percentage of (39%) among the fungal isolates and this may be due to its ability to resist difficult environmental conditions, bear wide thermal ranges, and produce enormous reproductive units that can resist difficult conditions. While *C. albicans* yeast represented the highest percentage of 65.8% among the isolated yeasts, this may be due to the widespread use of antibacterial drugs that alter the growth and livelihood of the natural flora and make it pathogenic organisms. The isolation of *C. albicans* yeast from clinical models of patients suffering from various diseases confirms the importance of this yeast as an important pathogenic cause in addition to *C. tropicalis*, *C. krusi* causes many diseases in the body[17,18,19]. The prevalence of infection with some causes may be due to its presence and its widespread in the environment and its possession of some virulence factors that enable it to cause infection[20,21].

Table 3: types of fungi isolated from patients according to the type of infection.

Fungal infection	Total Of number		Allergy		Tuberculosis		Bronchitis		Asthma		Pneumonia	
	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%	Number Of isolates	%
Fungal isolation												
A. terrus	12	12.1	3	25	4	33.3	3	25	2	16.6	0	0
A. flavus	6	6.1	2	33.3	4	66.6	0	0	0	14.2	0	0
A.fumigatus	17	17.1	10	58.8	5	29.4	2	5.8	1	5.8	0	0
A. altematae	3	3.0	0	0	3	100	0	0	0	0	0	0
Pencillium Spp.	9	9.1	5	55.5	4	44.4	0	0	0	0	0	0
Aeurobasidium	5	5.1	4	80	1	20	0	0	0	0	0	0
Rhizopus	2	2.0	1	50	1	50	0	0	0	0	0	0
Cladosporium	5	5.1	3	60	2	40	0	0	0	0	0	0
Mucor	2	2.0	1	50	1	50	0	0	0	0	0	0
A. niger	38	38.3	14	36.8	16	42.1	4	10.5	2	5.2	2	5.2
Total fungi isolates	99	70.7	43	43.4	41	41.4	8	8.1	5	5.1	2	2.0
Yeast isolates												
C.albicans	25	64.1	12	48	8	32	3	12	2	8	0	0
C. tropicalis	5	12.8	4	80	0	0	0	0	0	0	1	20
C. krusi	3	7.6	1	33.3	1	33.3	1	33.3	0	0	0	0
G. candidium	2	5.1	1	50	0	0	1	0	0	0	0	0
Rhodoteula	4	10.2	2	50	2	50	0	50	0	20	0	0
Total isolates Of yeasts	39	27.8	20	51.2	11	28.2	5	12.8	2	5.1	1	2.5
Total summation	140	100										

3- Immunological study:

The Effect of Respiratory Infection on the Phagocytic Activity of Polymorphonuclear Neutrophils (PMN).

The results showed a significant increase ($p < 0.05$) in the phagocytic activity of Polymorphonuclear Neutrophils (PMN) for age groups of people with respiratory infections compared to control groups. The highest percentage of nitro blue tetrazolium reduction appeared in the age group 51-60, where the average percentage of positive cells was 21,000 ± 2.094 compared to the control 11.857 + 1.431. The lowest percentage of pigment reduction that appeared in the age group11-20 was 13,200 + 1.652 compared to the control 10.9500 + 1.118, Table (4).

The current study indicated that respiratory tract infections stimulated the phagocytic activity of polymorphonuclear cells for all age groups. The NBT dye reduction test represents a cytochemical immunoassay that reveals the activity of macrophages involved in the process of phagocytosis, and it is known that neutrophils play a role in the phagocytic activity, which represents one of the non-specific immune mechanisms and is stimulated by the presence of foreign bodies[22].

Table 4: Percentage of PMNs positive for Nitoblue tetrazolium (NBT) dye test

Age group	Group	Percentage of polymorphonuclear cells positive for dye (NBT) (Average ± Standard deviation)
1-10	Control	15.823 ± 1.535
	Infection patient	*20.132 ± 1.847
11-20	Control	10.8400 ± 1.117
	Infection patient	*13.201 ± 1.651
21-30	Control	11.213 ± 1.22
	Infection patient	*17.370 ± 1.341
31-40	Control	9.024 ± 1.108
	Infection patient	*14.313 ± 1.157
41-50	Control	11.015 ± 2.371
	Infection patient	*17.441 ± 3.895
51-60	Control	11.856 ± 1.430
	Infection patient	*21.001 ± 2.093
61-70	Control	9.384 ± 1.024
	Infection patient	*13.601 ± 1.020

The mark * indicates the presence of significant differences under the level of significance ($p < 0.05$) compared to the control.

Conclusion

Respiratory diseases are considered serious diseases that affect all age groups and can lead, in most cases, to death. Most infections are caused by different types of fungi and bacteria. The current study indicated that respiratory infections stimulated the phagocytic activity of polymorphonuclear cells for all age groups. Therefore, early diagnosis and correct treatment have an important role in avoiding the negative results of respiratory diseases. We recommend conducting more studies on respiratory pathogens and their relationship to stimulating the immune system of infected patients to reduce their risk in the future.

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